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A Nested Case-Control Study of Dietary Factors and the Risk of Incident Cytological Abnormalities of the Cervix

L. Wideroff, N. Potischman, A. G. Glass, C. E. Greer, M. M. Manos, D. R. Scott, R. D. Burk, M. E. Sherman, S. Wacholder, and M. Schiffman

Abstract: *Several earlier case-control studies reported inverse associations of cervical squamous intraepithelial lesions (SIL) with high dietary or biomarker levels of carotenoids, folate, and vitamins C and E. However, most studies did not measure the primary causal factor, cancer-associated genital human papillomaviruses (HPV), now detected by sensitive viral DNA tests. This nested case-control study assessed whether high dietary intakes of these nutrients, plus zinc and vitamin A, reduced SIL risk in cancer-associated HPV DNA-positive women. Using a 60-item food-frequency questionnaire, nutrient estimates were obtained for 33 incident cases with high-grade lesions, 121 with low-grade lesions, 97 with equivocal SIL, and 806 cytologically normal controls sampled from a large prospective cohort study. Baseline cervicovaginal lavages were tested for HPV DNA by the polymerase chain reaction. Among DNA-positive cases* (n = 68) *and controls* (n = 69), *age-adjusted odds ratios (ORs) of SIL in the highest vs. the lowest nutrient quartiles were 1.4 [95% confidence interval (CI) = 0.5–4.2] for vitamin A, 0.6 (CI = 0.2–2.0) for β-carotene, 1.3 (CI = 0.4–3.6) for vitamin C, 1.0 (CI = 0.4–3.6) for vitamin E, 0.7 (CI = 0.3–2.1) for folate, and 0.8 (CI = 0.3–2.2) for zinc. ORs in HPV DNA-negative women approximated 1.0, with the exception of vitamin E (OR = 0.5, CI = 0.3–0.9). These results do not support a protective role for the above nutrients against low-grade or equivocal SIL, which constituted the majority of diagnoses in this study.*

Introduction

Although oncogenic human papillomaviruses (HPV) have been clearly established as the primary etiological agents in cervical neoplasia, most HPV infections clear spontaneously without leading to the diagnosis of any cervical cytological abnormality. The persistence of genital HPV infection, with the development of cytologically evident intraepithelial neoplasia, may be influenced by host and environmental cofactors that vary from one individual to another (1). Various etiological studies of risk factors for cervical neoplasia have examined the role of diet, hypothesizing that individuals with a relatively high dietary intake of certain nutrients have a reduced risk of developing intraepithelial and invasive lesions. Of particular interest are antioxidants, such as carotenoids, ascorbate, and tocopherols (2,3); regulators of DNA synthesis and repair, such as folate (4–6); mediators of cell growth and differentiation, such as vitamin A and the above antioxidants (7); and enzymatic constituents that are active in various metabolic pathways, such as zinc (8).

Published epidemiologic studies have fairly consistently reported an inverse association of vitamin C and carotenoids with preinvasive and invasive lesions of the cervix, whereas results have been equivocal for vitamin E and folate and generally negative for vitamin A (9). However, many of the studies designed to address the role of diet in cervical neoplasia were conducted before the advent of sensitive and specific laboratory methods for the detection of genital HPV infection, thus limiting their ability to assess nutritional risk factors while taking into account the critical role of HPV infection status. The objective of this study was to evaluate the association of nutritional factors with the risk of low- (LSIL) and high-grade squamous intraepithelial lesions (HSIL) of the cervix, using polymerase chain reaction (PCR) methods to define the presence or absence of genital HPV DNA. For completeness, cases of equivocal SIL [formally known as atypical squamous cells of undetermined significance (ASCUS)], defined after a rigorous pathology review, were also included. The main objective was to determine whether a higher intake of the micronutrients under study (i.e., carotenoids, folate, zinc, and vitamins A, C, and E)

L. Wideroff was, and N. Potischman, S. Wacholder, and M. Schiffman are, affiliated with the Epidemiology and Biostatistics Program, Division of Cancer Epidemiology and Genetics; L. Wideroff is affiliated with the Applied Research Branch, Division of Cancer Control and Population Science, National Cancer Institute, Bethesda, MD 20892. A. G. Glass and D. R. Scott are affiliated with the Kaiser Permanente Center for Health Research, Portland, OR 97227. C. E. Greer and M. M. Manos were affiliated with the Cetus Corporation, Emeryville, CA 94608; C. E. Greer is affiliated with the Chiron Corporation, Emeryville, CA 94608, and M. M. Manos with the Kaiser Research Institute, Oakland, CA 94611. R. D. Burk is affiliated with the Albert Einstein College of Medicine, Bronx, NY 10461. M. E. Sherman is affiliated with the Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD 21205.

was associated with a reduced risk of SIL, particularly in initially cytologically normal women who tested DNA-positive for cancer-associated HPV types at cohort enrollment.

Methods

Epidemiologic Methods

A prospective cohort study on the natural history of HPV infection and associated cervical SIL was conducted by the National Cancer Institute, in collaboration with Kaiser-Permanente clinical facilities in the Portland, OR area. The incidence cohort was established from a population of 17,654 clinic patients with no known history of SIL, who had normal routine screening results between April 1989 and November 1990. Follow-up continued until December 1994. As part of a nested case-control study of incident SIL, all cohort members who presented with an abnormal smear during the four- to five-year passive follow-up period were matched to approximately three controls who continued to be cytologically normal on the basis of age, follow-up time, enrollment clinic type (obstetrics/gynecology vs. health appraisal), enrollment cytology (completely negative vs. benign reactive atypia), and whether they returned a brief baseline questionnaire.

Cases who were identified at a screening visit during the follow-up period and their matched controls were interviewed about sexual behavior and additional life-style factors, reproductive and contraceptive history, sexually transmitted diseases, and other epidemiologic variables evaluated in the nested case-control study. Diagnoses of SIL were confirmed or excluded (approximately one-third) through independent review of the biopsies and/or abnormal smears by two expert pathologists, with discrepant diagnoses referred to a third reader. Diagnoses were based on the Bethesda system (10). Previous smears of the cases and controls from before cohort enrollment and during follow-up were included in the reviews to exclude prevalent cases. Cells for HPV DNA testing were collected by cervicovaginal lavage at cohort enrollment.

Nutritional Assessment

The nutritional component of the nested case-control study used the abbreviated Block food-frequency questionnaire (11), which was distributed to cases and controls at the diagnostic follow-up visit for self-administration. This optically scannable questionnaire measures frequency of intake and portion size for 60 food items and groups of similar items (i.e., apples and pears) plus intake of various types of supplements [multiple vitamins (Stress-Tabs type; therapeutic or Theragran type; One-a-Day type; or Centrum) and vitamins A, C, and E, calcium, or Dolomite]. Dosage was obtained for intake of calcium and vitamin C single supplements. Directions for completion of the questionnaire, together with some examples, were indicated on the front of the instrument, and study staff contacted subjects to retrieve information for missing food items that were key sources of nutrients of interest. Food-frequency data were converted to estimated units of nutrient intake using DIETSYS version 3.7 software (12). Quartiles were created for logistic regression analyses on the basis of the distribution of nutrient intakes in the controls.

HPV DNA Testing

Enrollment lavage samples were tested for HPV DNA by PCR in two laboratories. A consensus sequence in the L1 region of the viral genome was coamplified with a 268-bp human β-globulin fragment, which was included as a control for DNA integrity and amplification. In the first laboratory, PCR products were typed by dot-blot hybridization with type-specific probes for HPV types 6/11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, and 68 and novel types preliminarily identified as PAP155, PAP238A, PAP291, and W13B (13). In later testing at the second laboratory, remaining specimens were tested for the above types plus HPV types 2, 13, 32, 34, 61, 62, 64, 66, 67, 69, 70, and 72 and novel types AE2, AE7, and AE8 (14). DNA testing was conducted without knowledge of diagnosis or epidemiologic exposures, incorporating appropriate positive and negative controls, anticontamination measures, and repeat testing for reliability.

For this analysis, subjects were subsequently classified as HPV DNA-negative if viral DNA was not detected in their enrollment lavage specimen; as positive for a cancer-associated type if HPV type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, or 68 DNA was detected in their specimen (15); or as positive for a low-risk type (i.e., types not associated with cancer) if DNA from HPV type 2, 6, 11, 13, 26, 32, 34, 40, 42, 43, 44, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 72, PAP238A, W13B, PAP155, PAP291, AE2, AE7, or AE8 or an unidentified type was detected in their specimen without codetection of cancer-associated types.

Statistical Analysis

Of the 1,434 women selected for the dietary nested case-control study, 1,302 (90.8%) returned the questionnaire. However, 114 (8.8%) of the 1,302 women were determined to be ineligible as incident cases or controls after review of all their smears (average = 6/subject) indicated a history of SIL. An additional 51 women (3.9%) were ineligible because they did not have an enrollment HPV DNA test ($n = 20$) or because of questionable testing results (i.e., weak generic signal and negative for all type-specific probes, $n = 31$). Of the remaining 1,137 eligible women, 17 cases and 63 controls who reported eating fewer than three foods per day were excluded from the analysis because of questionable quality of their dietary intakes. Thus a total of 1,057 women remained in the dietary analysis, including 121 with LSIL, 33 with HSIL, 97 with equivocal SIL, and 806 controls.

For each micronutrient, age-adjusted (with age as a continuous variable), logarithmically transformed means of daily

intake were compared in cytopathology subgroups through analysis of covariance. Means were calculated on the basis of dietary intake alone and for nutrients with supplement data on the basis of total intake from diet and supplements. Means were further stratified by cytopathology and HPV DNA status. Adjustment for caloric intake did not alter the means and, therefore, was not done in the analysis presented here. Correlations between micronutrients were measured by Pearson correlation coefficients obtained in the control group.

For each micronutrient with supplement data, the odds ratios (ORs) and 95% confidence intervals (CI) of incident cytological abnormalities were calculated for increasing quartiles of micronutrient intake, using the lowest quartile as the referent category. Separate analyses were conducted for women who were HPV DNA-positive for cancer-associated types at enrollment (68 cases, 69 controls) and HPV DNA-negative women (146 cases, 683 controls); the small numbers of women who were DNA-positive solely for low-risk types (37 cases, 54 controls) precluded the calculation of stable risk estimates. Because relatively few cases and controls within matched sets were concordant in their HPV DNA status, an unmatched analysis was conducted using unconditional multivariate logistic regression to adjust for the matching variables, as well as for known risk factors of disease. The ORs were calculated including and excluding the cases with equivocal SIL. Separate ORs of LSIL and HSIL were not computed because of insufficient sample size in the HSIL subgroup.

Results

The mean age was 33 years in the control group, 36 years in cases diagnosed with equivocal SIL, 29 years in those with LSIL, and 33 years in those with HSIL. The median time to diagnosis was 794 days. Among the cases, 27.1% tested DNA-positive for cancer-associated HPV types at cohort enrollment in contrast to 8.6% of controls. An additional 14.7% of cases and 6.7% of controls were DNA-positive for other HPV types only; 58.2% of cases and 84.7% of controls were HPV DNA-negative.

Table 1 shows the estimated geometric mean daily intake, excluding supplement usage, of vitamins A, C, and E, folate, zinc, and five carotenoids (β-carotene, α-carotene, lycopene, lutein, and cryptoxanthin), adjusted for age and stratified by final diagnosis. Although nutrient means were slightly higher in the equivocal SIL cases than in controls, the differences were not statistically significant. Elevated means of α-carotene, β-carotene, and lycopene in HSIL cases were also not significant. Unexpectedly, the means of vitamins A and C, cryptoxanthin, and folate were significantly ($p < 0.05$) higher in the LSIL cases. Adjustment for calories did not notably alter the results.

Because differential usage of supplements between the diagnostic subgroups could influence these comparisons, the analysis was repeated incorporating supplement usage. Supplement data were not available for individual carotenoids with the exception of the multivitamin component β-carotene. Age-adjusted geometric means were further stratified by HPV DNA status at time of cohort enrollment to determine whether differences in mean intake between the cytopathology subgroups varied in HPV DNA-positive vs. HPV DNA-negative women (Table 2). DNA-positive women were further classified as positive for cancer-associated types or for other types only. Means are shown for all cases combined and for SIL cases excluding women diagnosed with equivocal SIL. With the exception of lower mean vitamin E intake in HPV DNA-negative cases than in controls, there were no statistically significant differences between cytopathology groups irrespective of enrollment HPV DNA status.

Pearson correlation coefficients in the control group between the nutrients (including supplement usage) and also

Table 1. Age-Adjusted Geometric Means (and 95% Confidence Intervals) of Daily Nutrient Intake[a] By Cytological Diagnosis[b–d]

Nutrient	Controls (n = 806)		ASCUS (n = 97)		LSIL (n = 121)		HSIL (n = 33)	
Vitamin A, IU	3,766*	(3,632–3,904)	3,993	(3,588–4,444)	4,244*	(3,858–4,668)	3,927	(3,278–4,705)
Carotenoids, μg								
α-Carotene	124.2	(114.8–134.3)	133.7	(106.0–168.6)	143.1	(116.4–176.0)	144.1	(97.4–213.2)
β-Carotene	967.6	(923.5–1,013.8)	1,069.7	(931.8–1,228.1)	1,083.9	(958.4–1,225.7)	1,059.4	(839.0–1,337.6)
Lycopene	719.0	(679.0–761.4)	781.2	(659.5–925.4)	812.0	(698.2–944.2)	858.0	(644.5–1,142.1)
Lutein	443.1	(416.1–471.8)	531.5	(441.5–639.9)	526.5	(446.2–621.1)	434.0	(317.2–593.8)
Cryptoxanthin	21.6*	(20.1–23.3)	21.8	(17.3–27.4)	26.0*	(21.2–32.0)	20.3	(13.8–30.0)
Vitamin C, mg	56.2*	(54.2–58.4)	59.3	(53.1–66.2)	65.5*	(59.4–72.2)	52.8	(43.8–63.6)
Vitamin E, α-TE	6.3	(6.1–6.5)	6.5	(5.8–7.2)	6.7	(6.2–7.4)	6.5	(5.4–7.8)
Folate, μg	176.9*	(171.0–183.0)	183.5	(166.0–202.9)	196.1*	(179.3–214.4)	169.9	(143.4–201.3)
Zinc, mg	7.7	(7.5–8.0)	8.1	(7.4–8.9)	8.2	(7.6–8.9)	7.3	(6.3–8.6)
Calories, kcal	1,253.7	(1,221.9–1,286.4)	1,252.3	(1,160.6–1,351.2)	1,302.8	(1,217.4–1,394.0)	1,238.8	(1,089.6–1,408.6)

a: Excludes supplement usage.

b: Values are means, with 95% confidence intervals in parentheses.

c: Abbreviations are as follows: ASCUS, atypical squamous cells of undetermined significance; LSIL and HSIL, low- and high-grade squamous intraepithelial lesions; α-TE, α-tocopherol equivalents.

d: Statistical significance of difference in means is as follows: *, $p < 0.05$.

Table 2. Age-Adjusted Geometric Means (and 95% Confidence Intervals) of Daily Nutrient Intake[a] By Cytopathology and Enrollment HPV DNA Status[b,c]

Nutrient	Controls (n = 806)[d]		All Cases (n = 251)[e]		LSIL and HSIL Only (n = 154)[f]	
Vitamin A, IU						
HPV DNA-negative	5,017	(4,775–5,272)	4,914	(4,408–5,477)	4,960	(4,292–5,732)
HPV DNA-positive						
Cancer-associated types	5,344	(4,559–6,263)	5,303	(4,519–6,222)	5,475	(4,526–6,623)
Other types	4,934	(4,157–5,855)	4,671	(3,690–5,912)	4,329	(3,204–5,849)
β-Carotene, μg						
HPV DNA-negative	1,251.1	(1,182.9–1,323.1)	1,256.5	(1,111.3–1,420.7)	1,230.0	(1,031.1–1,427.0)
HPV DNA-positive						
Cancer-associated types	1,352.1	(1,127.8–1,620.9)	1,426.5	(1,191.5–1,707.9)	1,453.3	(1,172.1–1,801.9)
Other types	1,095.3	(915.5–1,310.5)	1,223.9	(956.2–1,566.8)	1,113.3	(818.0–1,515.3)
Vitamin C, mg						
HPV DNA-negative	98.2	(90.9–106.0)	91.0	(76.8–107.7)	94.9	(75.7–119.0)
HPV DNA-positive						
Cancer-associated types	97.2	(76.8–123.2)	114.5	(90.3–145.2)	125.3	(95.0–165.2)
Other types	96.0	(73.3–125.8)	102.4	(70.6–148.5)	85.8	(54.1–136.0)
Vitamin E, α-TE						
HPV DNA-negative	11.4*	(10.6–12.2)	9.3*	(8.0–10.8)	9.3	(7.6–11.4)
HPV DNA-positive						
Cancer-associated types	12.3	(10.0–15.2)	12.0	(9.7–14.8)	12.1	(9.5–15.5)
Other types	11.0	(8.7–13.9)	12.1	(8.8–16.6)	10.4	(6.9–15.7)
Folate, μg						
HPV DNA-negative	257.8	(244.2–272.0)	248.7	(221.0–279.9)	242.2	(207.1–283.3)
HPV DNA-positive						
Cancer-associated types	286.3	(240.3–341.2)	266.4	(223.3–317.8)	281.4	(228.1–347.3)
Other types	247.2	(204.8–298.3)	267.8	(205.9–345.7)	252.9	(181.2–353.0)
Zinc, mg						
HPV DNA-negative	10.6	(10.1–11.1)	9.9	(8.9–11.0)	9.8	(8.5–11.2)
HPV DNA-positive						
Cancer-associated types	11.6	(10.6–13.6)	10.6	(9.1–12.4)	10.9	(9.0–13.1)
Other types	10.1	(8.4–11.9)	11.0	(8.7–13.9)	10.1	(7.4–13.6)

a: Includes supplement usage.
b: Values are means, with confidence intervals in parentheses.
c: Statistical significance of difference in means is as follows: *, $p \leq 0.05$.
d: Includes 683 human papillomavirus (HPV) DNA-negatives; 69 DNA-positives for cancer-associated types; 54 DNA-positives for other types.
e: Includes 146 HPV DNA-negatives; 68 DNA-positives for cancer-associated types; 37 DNA-positives for other types.
f: Includes 82 HPV DNA-negatives; 49 DNA-positives for cancer-associated types; 23 DNA-positives for other types.

daily caloric intake are shown in Table 3. Nutrient intake was not consistently correlated with caloric intake, yielding coefficients ranging from 0.0005 for vitamin C to 0.34 for zinc. However, correlations between the specific nutrients were generally strong, with all coefficients >0.40, except for vitamin C with β-carotene (0.27) and zinc (0.23). Correlations were similar when nutrient estimates excluded supplement usage (data not shown).

The age-adjusted ORs of incident cytological abnormalities (i.e., LSIL, HSIL, or ASCUS) by quartiles of mean daily nutrient intake, including supplement usage, are shown in Table 4 for women who tested HPV DNA-negative or DNA-positive for cancer-associated types at enrollment. Only one nutrient was entered per model, given the relatively high correlations observed between various nutrients.

Table 3. Pearson Correlation Coefficients of Total[a] Nutrients in Controls

	Vitamin A	β-Carotene	Vitamin C	Folate	Vitamin E	Zinc	Kilocalories
Vitamin A	1.00						
β-Carotene	0.75	1.00					
Vitamin C	0.51	0.27	1.00				
Folate	0.76	0.68	0.46	1.00			
Vitamin E	0.75	0.45	0.54	0.63	1.00		
Zinc	0.73	0.68	0.23	0.89	0.56	1.00	
Kilocalories	0.22	0.18	0.0005	0.21	0.06	0.34	1.00

a: Nutrient estimates (except calories) include supplement usage.

Table 4. Age-Adjusted Odds Ratios of Incident Cytological Abnormalities by Quartiles of Daily Nutrient Intake[a] in HPV DNA-Negative and Cancer-Associated HPV DNA-Positive Women[b]

	HPV DNA-Negative				Cancer-Associated HPV DNA-Positive			
	n				*n*			
Nutrient	Controls	Cases	OR	95% CI	Controls	Cases	OR	95% CI
Vitamin A, IU								
≤2,793	23	141	1.0		10	14	1.0	
2,794–4,673	46	185	1.5	0.8–2.6	20	14	1.9	0.6–5.5
4,674–8,043	44	174	1.5	0.8–2.6	17	23	1.0	0.3–2.8
≥8,044	33	183	1.0	0.6–1.8	21	18	1.4	0.5–4.2
β-Carotene, μg								
≤666	28	143	1.0		11	9	1.0	
667–1,214	44	177	1.3	0.8–2.2	15	18	0.6	0.2–2.0
1,215–2,124	37	182	1.0	0.6–1.8	21	20	0.8	0.2–2.3
≥2,125	37	181	1.0	0.6–1.7	21	22	0.6	0.2–2.0
Vitamin C, mg								
≤44	23	141	1.0		11	14	1.0	
45–75	43	185	1.4	0.8–2.4	21	15	1.9	0.7–5.6
76–147	49	182	1.6	0.9–2.8	18	23	1.0	0.4–2.8
≥148	31	175	1.0	0.6–1.8	18	17	1.3	0.4–3.6
Vitamin E, α-TE								
≤5	33	137	1.0		16	14	1.0	
6–8	38	190	0.8	0.5–1.4	12	14	0.8	0.2–2.2
9–24	51	180	1.2	0.7–1.9	18	23	0.6	0.2–1.7
≥25	24	176	0.5	0.3–0.9	22	18	1.0	0.4–2.6
Folate, μg								
≤135	23	140	1.0		13	11	1.0	
136–219	47	180	1.6	0.9–3.0	17	20	0.7	0.2–1.9
220–474	46	183	1.5	0.9–2.6	16	16	0.7	0.2–2.2
≥475	30	180	1.0	0.5–1.7	22	22	0.7	0.3–2.1
Zinc, mg								
≤6	27	133	1.0		14	14	1.0	
7–9	35	185	1.0	0.6–1.7	15	14	1.0	0.3–2.9
10–17	54	187	1.5	0.9–2.5	20	19	1.0	0.4–2.9
≥18	30	178	0.8	0.4–1.5	19	22	0.8	0.3–2.2

a: Includes supplement usage.
b: HPV DNA-negative subgroup includes 146 cases and 683 controls; cancer-associated HPV DNA-positive subgroup includes 68 cases and 69 controls. CI, confidence interval.

Among the 68 cases and 69 controls who tested DNA-positive, a slightly decreased risk was suggested for higher levels of β-carotene and folate and for intermediate levels of vitamin E. However, 95% CI around the risk estimates of all six micronutrients included 1.0, and trends of decreasing risk with increasing intake were not observed. Among the HPV DNA-negative cases (n = 146) and controls (n = 683), higher intakes of micronutrients were not inversely associated with disease, and, unexpectedly, modest increases in risk were observed in some of the intermediate quartiles relative to the lowest. However, the 95% CIs around the elevated ORs included 1.0. Further adjustment for smoking, race, income, oral contraceptive use, parity, pregnancy status at the time of response to the diet questionnaire, duration of follow-up, or matching variables other than age did not alter the relationship of nutrient intake to disease. Results were also not altered after adjustment in the HPV DNA-negative group for lifetime number of sex partners, a surrogate for HPV infection not detected at enrollment. Exclusion of equivocal and/or HSIL cases had no notable effect on the risk estimates.

Discussion

On the basis of nutrient estimates derived from a 60-item food-frequency questionnaire, cancer-associated HPV DNA-positive women in the highest quartiles of vitamins A, C, and E intake were not at a significantly reduced risk of incident squamous cell lesions of the cervix relative to women in the lowest quartile (Table 4). Although the ORs suggested a 20–40% reduction in risk for women in the highest quartiles of β-carotene, folate, and zinc intake and also in intermediate quartiles of β-carotene, folate, and vitamin E intake, 95% CI included 1.0, and therefore chance cannot be ruled out as an explanation for these associations. These results suggest that in the presence of viral DNA the above nutrients are not important protective cofactors

against the development of low-grade or equivocal lesions, which comprised the majority of lesions in this study. The small number of incident high-grade lesions precluded the estimation of ORs of HSIL by nutrient intake in cancer-associated HPV DNA-positive women. On the basis of these small numbers, HSIL cases did not show lower age-adjusted means of daily intake than controls (Table 1), which again does not support a protective effect.

The stratum that was HPV DNA-negative at baseline included women who remained DNA-negative throughout the study as well as women who became DNA-positive during follow-up. ORs in this heterogeneous stratum were calculated separately so as not to influence risk estimates of SIL in women who were DNA-positive for cancer-associated HPV types at enrollment. The ORs and 95% CI did not show a reduced risk of incident squamous cell lesions for HPV DNA-negative women in the highest quartiles of vitamins A and C, β-carotene, folate, and zinc, suggesting that these nutrients are not protective in the absence of viral DNA detection.

However, HPV DNA-negative cases did have a statistically significant lower mean daily intake of vitamin E than controls when supplement usage was taken into account (Table 2), although significant differences between the cytopathology groups solely on the basis of foods were not observed (Table 1). HPV DNA-negative women in the highest quartile of vitamin E intake showed a 50% reduced risk relative to those in the lowest quartile (95% CI = 0.3–0.9); ORs in the second and third quartiles varied (Table 4). Although these ORs suggest a potential protective effect at the highest level of vitamin E intake, a trend was not observed and small numbers make interpretation difficult.

Moderately elevated ORs, with lower 95% CI approaching 1.0, were observed in intermediate quartiles of several micronutrients, including vitamins A and C and folate. Case-control studies that rely on self-report are frequently prone to differential and nondifferential misclassification. Anomalous findings such as these direct associations may reflect a combination of differential and nondifferential misclassification, the effects of which were possibly exacerbated by the small number of cases in this study. Alternatively, these risk estimates may represent chance or real statistical associations.

Other case-control studies examining dietary factors in relationship to cervical intraepithelial lesions of varying severity have reported equivocal results. In one study, risk estimates <1.0 were reported for high vs. low intake of carotenoids and reduced risk for high serologic levels of individual carotenoids other than lutein (16). Studies examining total carotenoids have reported nonsignificant decreases using serologic measurements (17,18), a significant direct association based on dietary intake (19), or no association (20). The few case-control studies to examine associations of folate and vitamins C and E with SIL of varying severity have reported a protective effect for high dietary vitamin C intake (19,21), high dietary or blood levels of folate (17,21), and high serologic levels of α-tocopherol (22). Case-control studies of vitamin A and SIL have generally not reported a protective effect (9).

Case-control studies that were restricted to severe preinvasive cervical lesions (e.g., carcinoma *in situ*) have generally reported decreased risks for high vs. low dietary vitamin C, which were marginally or not statistically significant, whereas results for dietary or serologic carotenoids and folate have been equivocal (18,23–25). A moderate, nonsignificant protective effect of dietary vitamin E has been reported (24) but not confirmed in a serologic study (26). In contrast to studies of carcinoma *in situ* or of varying disease severity, the results of the present study generally do not support a protective effect for diet in the occurrence of incident, mainly low-grade, cervical lesions in the presence or absence of genital HPV DNA detection.

Previous case-control studies that reported protective associations of diet and SIL have mainly relied on sexual behavior surrogate variables to adjust for HPV infection. However, the effects of measurement error (e.g., inadequate measurement of HPV status) must be considered in the interpretation of these associations (27). One major advantage of this study was the testing of cervicovaginal lavage specimens for a broad spectrum of genital HPV types, yielding detailed exposure classification of this strong primary risk factor and, consequently, permitting evaluation of viral-diet interactions.

Another major advantage was the extensive effort undertaken to correctly classify study participants with respect to incident case status. Thus the rigorous and reliable review, not only of new smears or biopsies but of preexisting ones as well, permitted identification and elimination of prevalent and recurring cases. Inconsistencies with the results of previous studies may be due to their inclusion of more cases with high-grade lesions or of cases with prevalent SIL. Because of the true incident nature of the cases and the low prevalence of genital HPV DNA in cytologically normal controls, the sample size of the cancer-associated HPV DNA-positive subgroup was somewhat limited, thus limiting statistical power and precluding stratification by diagnosis (i.e., LSIL, HSIL) or by effect modifiers, such as smoking, in the regression analysis.

In conclusion, the risk of incident cytological abnormalities of the cervix in cancer-associated HPV DNA-positive women was not found to decrease with increased intake of vitamins A, C, or E, β-carotene, folate, or zinc. In addition, cases did not exhibit a low mean daily intake of various other carotenoids relative to controls. This does not exclude, however, a role for dietary cofactors in the progression of low-grade lesions or latent HPV infection to HSIL. Although the subjects in this study were sampled from a large prospective cohort, the number of women diagnosed with incident HSIL was small, which precluded the use of multivariate regression analysis to assess dietary cofactors in this cytopathological subgroup. Future research to elucidate the role of various micronutrients in the development of HSIL and in the establishment of persistent HPV infection

may be valuable. Additional analyses of SIL based on micronutrient biomarkers, in addition to food-frequency data, may be useful for examining associations independent of the potential effects of measurement error related to dietary self-reports.

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